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Violent Phenotype in SAL Mice is Inflexible and Fixed in Adulthood

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Violence was shown to be qualitatively different from functional hyper-aggression in mice selected for high aggression namely Short Attack Latency (SAL), Turku Aggressive (TA) and North Carolina (NC900) strains. This study aimed at investigating whether this adulthood violent phenotype as seen previously in the SAL mice is *fixed* and hence behaviorally inflexible right from day 1 of the experiment or *consequential*, i.e., subject to gradual change from functional aggression to violence. The functionally hyper-aggressive strains namely TA and NC900 strains served as controls for the study. Methodologically, behavioral (in)flexibility was studied using the overall sequential *structure* of agonistic behavior. In particular, intra-individual variations in the overall agonistic behavior as well as offensive, pre- and post-offensive behavior transitions, directly related to the resident–intruder interactions were investigated. The SAL mice showed the least intra-individual variation in their overall sequential agonistic structure as well as a *fixed* offense-oriented agonistic behavior of highest magnitude when compared with the other strains. Additionally, the pre- and post-offensive transitions were most salient in the functionally hyper-aggressive TA and NC900 strains, whereas virtually absent in the SAL mice. Thus, the violent behavior of the adult SAL mice is behaviorally inflexible or *fixed*, whereas the functionally hyper-aggressive behavior of the adult TA and NC900 mice is behaviorally flexible and constantly adaptive to the opponent behavior, over 3 days of repeated resident–intruder interaction. Aggr. Behav. 35:430–436, 2009. © 2009 Wiley-Liss, Inc.

Keywords: violence; aggression; inflexibility; mouse; SAL

INTRODUCTION

Violence is a global problem addressed in detail by the WHO [Krug et al., 2002]; yet at a biological level, there is a paucity of research when it comes to the investigation of the causes and the underlying mechanisms behind violence. These studies require good animal models that reflect both violence and functional aggression, the latter of which is integral in the procurement of resources and ranking in the wild. An extensive ethological comparison of several genetic selection strains of mice revealed that short attack latency (SAL) mice are predisposed to violence when compared with the other strains namely Turku Aggressive (TA) and North Carolina (NC900) [Natarajan et al., 2009].

Briefly, the SAL mice were shown to display a highly limited agonistic behavioral repertoire, which was primarily offense-oriented when it comes to an unfamiliar male opponent in the familiar environment. SAL mice also attack anesthetized opponents in neutral environments [Natarajan et al., 2009]. The SAL mice additionally attack their female

cage-mates in particular after cage cleaning or after repeated inter-male interactions in the resident–intruder paradigm [Benus et al., 1990]. This indiscriminate behavior was clearly different from the two other high-aggression mouse strains in that the latter displayed a rich agonistic repertoire of behaviors and aimed at neither the anesthetized male opponents nor their female cage-mates [Caramaschi et al., 2008; Natarajan et al., 2009].

A large proportion of SAL mice routinely attacked unfamiliar male opponents upon repeated interactions [Benus et al., 1990; Caramaschi et al., 2008]. These intense agonistic interactions are relatively insensitive not only to the docile opponent but also to human interferences after the tests

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[personal observations]. Repeated interactions and/or indirect exposure to aggressive encounters in general are known to induce uninhibited aggression in animals as well as humans [de Boer and Koolhaas, 2005; Kudryavtseva, 2000; Kudryavtseva et al., 2000; McSweeney and Swindell, 2002; Winslow and Camacho, 1995; Winslow and Miczek, 1983]. The SAL mouse might therefore be considered an excellent animal model for pathological aggression or violence.

The next logical question that follows is to characterize the temporal dynamics of aggressive behavior in these mice. It is possible that violence is already fixed much earlier, during the time of adolescence or much earlier or even biologically driven and hence manifests as a *fixed/inflexible* behavior in adulthood or driven gradually from a functional form of aggression to violence owing to experiences and hence *consequential* in adulthood. This issue was addressed using brief repeated resident–intruder interactions over a period of three successive days.

Two straightforward outcomes can be anticipated with repeated interactions. First, a *fixed violent* phenotype with exclusive offense-oriented behaviors of high and unchanging magnitudes across tests at adulthood, which is insensitive to environmental cues and contexts. Second, an initially behaviorally flexible and rich agonistic repertoire that concomitantly decreases with time and thus becomes a *consequential violent* phenotype in adulthood.

Earlier, it was shown that the quantitative study of offensive behaviors in terms of duration and frequency was limited in its ability in delineating violence from functional aggression [Natarajan et al., 2009]. However, the analysis of behavioral transition matrices through behavioral kinetograms proved invaluable for this objective. Behavioral transition matrices refer to a frequency grid of transitions of several behaviors from behavior A to behavior B for any single animal or group of animals of the same strain for example. The key difference between aggression and violence was shown to be the differential detailed *structure* of the agonistic interaction. In order to investigate the nature of violence in SAL mice, this study made use of the same *structure* analysis of the agonistic behavior of high-aggression mouse strains namely NC900, TA and SAL over three successive days of repeated interaction with a docile opponent.

The first approach is to analyze the overall transition frequency matrices of all resident high-aggressive mice for intra-individual variation within each strain across all 3 days of repeated interaction.

A potential *fixed violent* phenotype can be anticipated to show the least intra-individual variation in the overall sequential structure of agonistic behavior when compared with a *consequential violent* irrespective of the time of successive interactions with an opponent. Further differences in flexibility between the *fixed violent* and *consequential violent* phenotypes were assessed using a detailed analysis of the microstructure of specific agonistic components namely the pre-, post- and offensive transitions of the resident.

MATERIALS AND METHODS

Study Species

High-aggression male mice ($n = 8$ per strain) aged 3–4 months from three different genetic selection lines namely SAL, TA, NC900 were considered for the behavior analysis. A detailed description of these animals and their selection, the resident–intruder paradigm used for this study is available elsewhere [Natarajan et al., 2009]. The opponents used for this study was a docile strain Mas-Gro. Both the animal care and the experimental design were approved by the Institutional Animal Care and Use Committee, University of Groningen [D4328A].

Briefly, the resident mice were bred and kept in familiar groups until weaning (3 weeks after birth), then co-housed with a female of the same line in Makrolon Type II cages (375 cm²). The litters were culled periodically. The mice were fed ad libitum on standard pellets (AMII, ABDiets, Woerden, The Netherlands) and water with low chloride content. They were exposed to a reverse light–dark cycle of 12 hr shifting at 00:30 hr. Each cage was provided with sawdust bedding, shredded paper (*envirodry*, the Netherlands), nesting and cardboard tubing enrichment materials. Room temperatures were maintained at $22 \pm 2^\circ\text{C}$.

Data Collection and Analysis

Figure 1 shows the behavioral design of the experiment. Inter-male interactions were preceded by separating the female of the pair, 1 hr before the

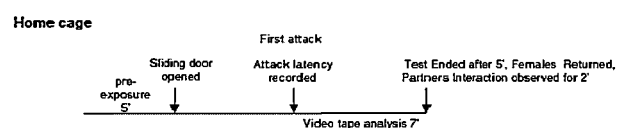


Fig. 1. Illustration of the experimental design used for a single test. The same was repeated for three successive days. For further experimental design details, refer Natarajan et al. [2009].

lights went off. The male mouse was allowed to retain one-half of the cage by introducing a sliding door, which separated the cage into two halves without limiting access to food or water. Five minutes before the test, the male opponent was introduced into the unoccupied half of the cage. The perforated sliding door allowed only sensory contact and prevented any direct physical contact between the males. The sliding door was then removed and the direct inter-male interaction was recorded. The interaction was allowed to last for 5 min after the first attack. When the resident failed to attack within the first 5 min of testing, the attack latency time was recorded as 300 and the test was terminated. The opponent was then removed from the cage and the resident's female partner was reintroduced and observed for a period of 2 min. This procedure was repeated for 3 successive days. The resident mice were allowed to combat with a new opponent on all 3 days and hence the effects of familiarity on the inter-male interaction were avoided. The following behaviors were scored on tapes and quantified: digging, nonsocial exploration (explore the cage), social exploration (approach, investigation—crawl over, crawl under, follow, allo-groom, head groom, investigate, nose sniffing), immobility, resting, body care (self grooming, wash, shake, scratch), feeding (drink/eat), attack (lunge, attack), chase (charge), threat (aggressive groom, sideways, offensive, upright offensive, tail rattle), defense [Koolhaas et al., 1980]. The quantification of these behaviors was carried out at 1/5th of the normal speed using Observer Pro 5.0 (Noldus BV, Wageningen, the Netherlands). This study assessed the frequency-based behavioral transition matrices from the observed sequence of behaviors for each subject per day. The matrices were obtained using lag-sequential analysis from the Observer Pro 5.0 software.

To allow a direct comparison between tests and strains, the overall behavioral transition matrices of all mice were normalized to yield comparable matrix totals regardless of strains and tests. Subsequently the adjusted residuals for each transition frequency matrix were obtained using MatMan software version 1.1.4 [Noldus Information Technology, Wageningen, The Netherlands; de Vries et al., 1993a]. These residual matrices in turn were used for assessing matrix-wide intra-individual variations as well as for comparing specific behavioral transitions between the three strains and tests. Adjusted residuals reflect the magnitude of the concerned transition in any given behavioral transition matrix. A positive residual means that the transition occurs more frequently than expected by chance conditional upon the row

and column totals. A negative residual indicates that the transition occurs less frequently than expected by chance [van Hooff, 1982].

Statistics

The intra-individual/between-test comparisons were based on Kendall's tau row-wise matrix correlations [de Vries, 1993b] matrices for each day calculated for each individual mouse within each strain. The individual tau correlation values for each strain were analyzed further using paired-samples *t*-tests between days.

Pre-, post-offensive transitions and the transitions within the offensive interaction were analyzed separately across strains and days. These transitions include: *social exploration* to *threat*; *social exploration* to *withdrawal* (classified under pre-offensive transitions); *threat* to *attack*; *attack* to *chase*; *chase* to *threat* (offensive transitions); *chase*/ *attack* to *withdrawal*/ *approach*—*withdrawal*; *withdrawal*/ *approach*—*withdrawal* to *nonsocial exploration*/ *immobility* (post-offensive transitions). Their reverse transitions were also considered for the analysis.

Changes over subsequent tests (i.e., days) on the above residuals measures were analyzed with ANOVA for repeated measurements with 'day' as a within-subject factor (3 levels: Days 1, 2, 3) and 'strain' (3 strains: SAL, TA and NC900) as between-subject factor. *Post-hoc* comparisons for the "strain" effects were analyzed using Tukey's HSD. Significant "day" \times "strain" effects were analyzed using linear contrasts. Significant decline or rise in the magnitude of the agonistic transitions was analyzed by linear contrasts across the three tests. To control chance capitalization the critical probability level for these linear contrasts was set at .016 ($= .05/3$). All tests were two-tailed with alpha set to .05. Any transitions with adjusted residual means less than zero for all mouse strains were not considered for the statistical analysis. Exceptions were made when at least one of the mouse strains displayed the concerned behavioral transition of magnitudes greater than one. All tests were done using SPSS 16.0 version.

RESULTS

Intra-Individual Variation in Overall Frequency Based Behavioral Transition Matrices

Intra-individual dyadic correlations between individual matrices of adjusted residuals were analyzed to assess for behavioral invariance across

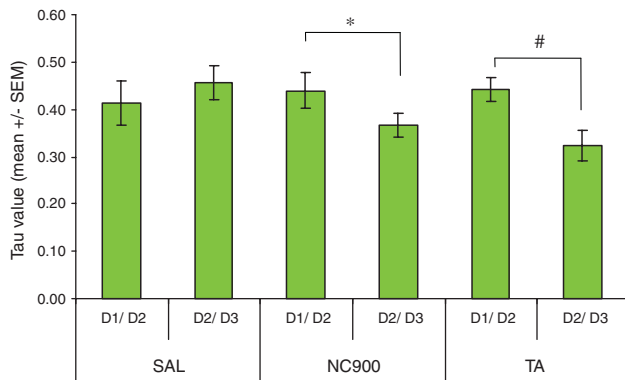


Fig. 2. Representation of the intra-individual variation in SAL, NC900 and TA mouse strains. Paired-samples *t*-tests between days D1 and D2; D2 and D3 were carried out in all strains. The TA and NC900 strains showed significant intra-individual changes across days, whereas SAL strain failed to show any. Data are plotted as mean \pm SEM. Significant “*P*” values are represented as $P < 0.05$ *; $P < 0.01$ #.

individuals between day 1 and day 2 and between day 2 and day 3 (Fig. 2). A high correlation between two transition matrices indicates a strong similarity in the overall sequential structure of the mouse’s observed behavior on the two consecutive days.

The SAL mice showed no significant difference between the correlations (day 1–day 2 correlations: mean = .41; day 2–day 3 correlations: mean = .45; paired-samples *t*-test: $t_{(7)} = -0.59$, NS). NC900 showed a significant decline (day1–day2 correlations: mean = 0.44; day2–day3 correlations: mean = .36; paired-samples *t*-test: $t_{(7)} = 2.37$, $P < .05$). TA showed an even stronger decline (day1–day2 correlations: mean = .44; day2–day3 correlations: mean = .32; paired-samples *t*-test: $t_{(7)} = 3.73$, $P < .01$). Thus, SAL scored the highest intra-individual test-to-test correlations suggestive of a *fixed* phenotype.

Inter-Strain Comparison of Aggressive Microstructure

The adjusted residuals of transition frequencies pertinent to pre-, post- and offensive behaviors were analyzed in detail between the strains. Only those transitions of appreciable magnitude and those that were found to yield statistically significant results are reported.

Pre-Offensive Transitions

Social exploration \rightarrow threat. A repeated measures ANOVA revealed a significant “strain” ($F_{(2,21)} = 5.92$; $P < .01$) and “day \times strain” ($F_{(4,42)} = 4.19$; $P < .01$) effect for this transition. *Post hoc* analysis with the strains reveal NC900 strain display this transition significantly higher than

the SAL mice ($P < .01$) and slightly higher than the TA mice ($P = .049$). The likelihood that *social exploration* is followed by *threat* was thus strongest in the NC900 strain with a maximum magnitude of four on day 3 (Fig. 3a). TA mice in particular, showed a significant linear decline across days ($P < .01$) with contrasts analysis. The transitions in the TA strain were comparable to NC900 strain on day 1 but resembled the SAL strain on day 3. SAL displayed the lowest and unchanging residuals of a magnitude less than two.

Threat \rightarrow withdrawal/ approach-withdrawal. With respect to transitions from *Threat* to *Withdrawal*, a “day” ($F_{(2,42)} = 3.81$; $P < .05$) and “strain” ($F_{(2,21)} = 5.42$; $P < .05$) effect was found. No significant interaction effect was found. *Post hoc* analysis reveals a significantly lower magnitude of this transition in the NC900 strain (figure not shown) when compared with the TA strain ($P < .05$) and only marginally lower than the SAL strain ($P = .054$).

With respect to *Threat \rightarrow Approach-withdrawal*, a significant “strain” ($F_{(2,21)} = 8.56$; $P < .005$) and “daystrain” ($F_{(4,42)} = 3.02$; $P < .05$) effect was found. *Post hoc* analysis for the “strain” effect reveals that TA display significantly lower magnitude of this transition when compared with both NC900 ($P < .01$) and SAL strains ($P < .005$). However, no significant linear increase or decrease was seen for each of the three strains across tests (figure not shown).

Offensive Transitions

Threat \rightarrow attack. A repeated measures ANOVA on this transition revealed a marginal effect of “day” ($F_{(2,42)} = 3.21$; $P = .05$) and a significant “strain” ($F_{(2,21)} = 12.55$; $P < .001$) effect. No significant interaction effect was found. *Post hoc* analysis reveal that NC900 display a higher magnitude of this transition than the TA strain ($P < .05$) and the SAL strain ($P < .001$). The latter was owing to the lowest and unchanging transition magnitudes displayed by SAL (less than two/ Fig. 3b).

Attack \leftrightarrow chase. A repeated measures ANOVA on this transition revealed a significant “strain” ($F_{(2,21)} = 6.41$; $P < .01$) effect. In particular, the SAL mice were distinctly higher than the TA mice ($P < .001$) and marginally higher than the NC900 mice ($P = .055$). No significant “day” or “day \times strain” interaction effects were found. A significant decline across the tests was found for TA ($P < .01$) (Fig. 3c).

Repeated measures ANOVA on the reverse transition from *chase* to *attack* revealed again a significant strain-specific effect ($F_{(2,21)} = 41.59$;

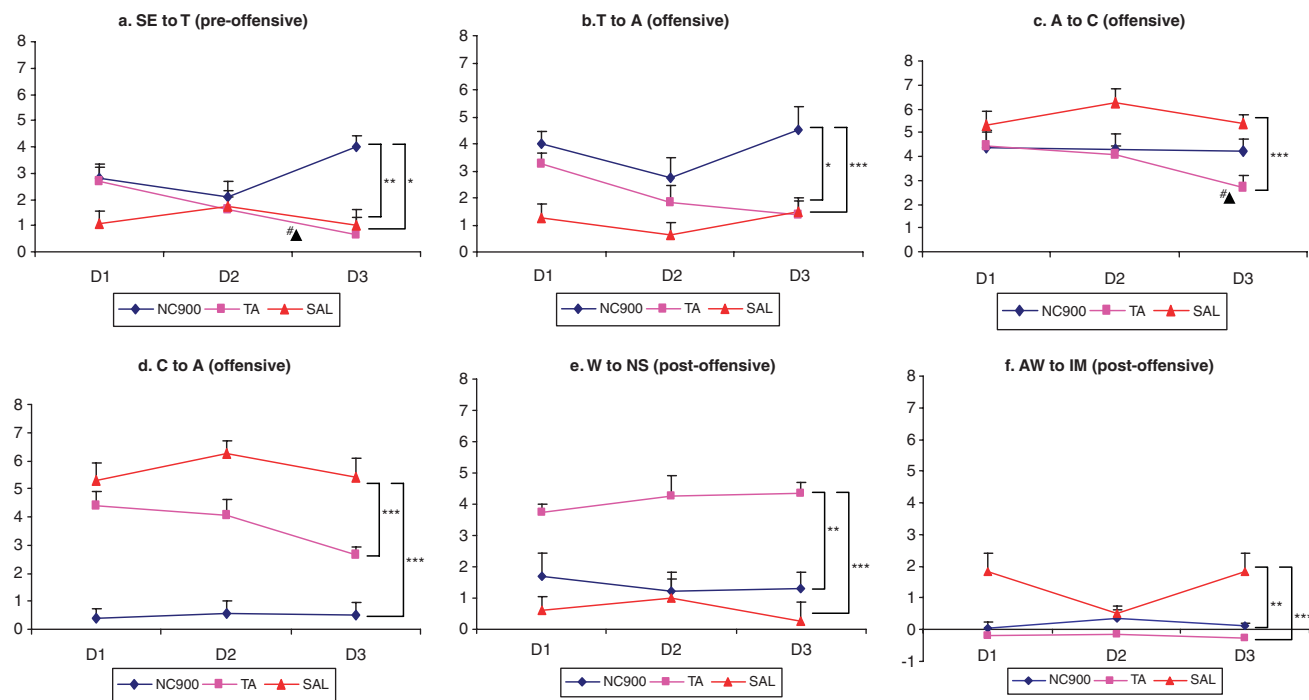


Fig. 3. (a-f) represents significant agonistic behavioral components pertinent to pre-, post- and offensive behavioral transitions for all high-aggressive mouse strains. The x-axis represents the days of testing and the y-axis represents the mean adjusted residuals \pm SEM for the concerned transition. Significant "strain" transitions are represented as $P < 0.05^*$; $P < 0.01^{**}$; $P < 0.001^{***}$. Significant linear contrasts are indicated by #.

$P < .001$) with no "day" or interaction effects. *Post hoc* analysis reveal that the SAL displayed the highest magnitude of this transition than any other strains ($P < 0.001$) as seen from Figure 3d.

Post-Offensive Transitions

Post-offensive transitions are dealt in two parts (1) transitions from *attack*, *chase* to *withdrawal*, *approach-withdrawal* behaviors (2) transitions from *withdrawal*, *approach-withdrawal* behaviors to *non-social exploration*, *immobility*.

Attack \rightarrow approach-withdrawal. A significant "strain" effect was found ($F_{(2, 21)} = 9.43$; $P = .001$) for this transition with no other main or interaction effects (figure not shown). The NC900 strain was markedly higher by magnitude across all 3 test days than the TA strain ($P = .001$).

Chase \rightarrow withdrawal/ approach-withdrawal. A significant "strain" effect was found for both the transitions *chase* to *withdrawal* ($F_{(2, 21)} = 5.12$; $P < .05$) and *chase* to *approach-withdrawal* ($F_{(2, 21)} = 25.00$; $P < .001$). No other main or interaction effects were found. *Post hoc* analysis for the former transition revealed slightly higher magnitude in NC900 strain when compared with the TA strain ($P < .05$). The *chase* to *approach-withdrawal* transition (figure not shown) was distinctly higher by magnitude in NC900 strain

when compared with both TA and SAL strains ($P < .001$).

Withdrawal \rightarrow nonsocial exploration. Repeated measures ANOVA reveal strong "strain" effects ($F_{(2, 21)} = 14.59$; $P < .001$). No "day" or "day \times strain" effects were seen. *Post hoc* analysis revealed TA mice show higher magnitudes of this transition than NC900 ($P < .005$) and SAL ($P < .001$) strains (Fig. 3e).

Approach-withdrawal \rightarrow immobility. Repeated measures ANOVA revealed a significant "strain" ($F_{(2, 21)} = 10.63$; $P = .001$) and "day \times strain" ($F_{(4, 42)} = 4.78$; $P < .01$) effect. *Post hoc* analysis for the "strain" effect revealed SAL display this transition significantly higher than NC900 ($P < .01$) and TA ($P = .001$) strains. The other strains showed an unchanging nature of this transition with poor magnitudes close to zero. No significant linear decline or rise in transitions was found for each of the strains (Fig. 3f).

The reverse transitions apart from *Attack* \leftrightarrow *Chase* were found to be of magnitudes lesser than or equal to zero and hence were not considered for the statistical analysis.

DISCUSSION

This study was aimed at whether the adult SAL mouse display violence head on from the first

aggressive confrontation onwards (*fixed* violence) or gradually (*consequential* violence) over repeated interactions with a docile male opponent in a resident–intruder paradigm. The following were the key findings of this study.

As anticipated, the SAL mice showed the least intra-individual variation compared with TA and NC900 strains, suggestive of SAL-specific constancy in an agonistic setup. The SAL male mice thus displayed an overall stable sequential structure in their agonistic behaviors over tests. However, this constancy observed in the SAL mice need not necessarily reflect a *fixed* violent nature. Hence, a more detailed analysis of this difference in intra-individual variation was aimed at the aggressive micro-structure; in particular the behavioral transitions that were reflective of direct resident–intruder interactions.

With respect to pre-offensive transitions, SAL showed invariant pre-offensive transitions of low magnitudes. TA, however, showed a decline in pre-offensive transitions suggestive of their declining flexibility with tests. NC900 strain showed a tendency to either increase or maintain fixed pre-offensive transitions higher than SAL or TA strains.

The offensive transitions were most diverse in the SAL strain than the others. The transition from chase to attack for instance is seen in SAL and TA strains while NC900 strain fails to show this transition. Further the TA strain shows a decline with days. Overall, these transitions were highly invariant and of highest magnitude than any other agonistic transitions/strains considered right from day 1. Although invariant, the transitions shown by the NC900 mice were of moderate magnitude, when compared with the SAL strain. The TA strain on the contrary showed a decline in the offensive transitions with tests.

Post-offensive transitions (other than to immobility) were found to be invariant and of high magnitudes in both the NC900 and the TA strain when compared with the SAL strain. High and stable transitions from *withdrawal/ approach–withdrawal* to *immobility* were found to be SAL-specific, which presumably reflects the physical exhaustion of the animal.

Direct resident–intruder related behavioral transitions were considered bi-directionally to assess whether there was a specific bias toward offensive behaviors. No significant reverse transitions were observed excepting for *Attack* ↔ *Chase*. Thus, the nature of agonistic interaction proceeded from pre-offensive to offensive and finally to post-offensive transitions in all these strains. However, the

agonistic repertoire specific patterns as described above clearly differed in the SAL mice when compared with the other mouse strains.

To summarize, the SAL mice showed a highly limited yet consistent offense-oriented agonistic repertoire right from day 1, in comparison to the other strains. This demonstrates the inflexible and *fixed* nature of violence in the SAL male given an agonistic setting. By contrast, despite displaying invariance in most transitions, the NC900 strain showed a rich agonistic repertoire inclusive of pre-, post- and offensive transitions. Additionally, the offensive transitions were less diverse and of moderate magnitudes in the NC900. These patterns thus reflect a stable but *flexible* aggressive phenotype in the NC900 strain. The TA strain showed an overall tendency to reduce their pre- and offensive transitions while maintaining their post-offensive transitions at an appreciable magnitude, thus suggestive of a *flexible* and functionally aggressive phenotype, which is adaptive in its agonistic responses with respect to the opponent. Agonistic interactions beyond the currently used three tests may be required to establish any transitions from functional aggression to *consequential violence* concretely, if any, in both the TA and the NC900 strains.

Given the lack of availability of original mouse populations, this study could not employ these controls for each selection line. Although the low-aggressive counterparts could have been used as a control, their failure to show sufficiently high frequency-based behavioral transitions made them less conspicuous control candidates for these analyses. However, both TA and NC900 strains were used as controls for the violent SAL mice in this study. These high-aggression lines in general showed comparable magnitudes of offensive durations, frequencies and frequency-based behavioral transitions, which make them an appropriate control from a methodological viewpoint [Natarajan et al., 2009].

Behavioral inflexibility in the SAL mice is not new and has already been shown in a number of nonsocial contexts as well [Benus et al., 1990]. For instance, the SAL mice have been shown to perform stereotypically in a Hebb–Williams maze both during training as well as during the test with altered intra- and extra-maze configurations. This high-aggression selection line failed to show any changes with both the latency to run with altered cues as well as the number of errors made during the runs toward the goal box. Moreover, SAL mice were reported to show the largest errors in a reversal-learning paradigm in which the mice had access for

food through only one arm of the Y-shaped living structure [Benus et al., 1991a]. The SAL mice have also been shown to display stereotypy behaviors upon apomorphine challenges than its low-aggressive counterparts namely the LAL mice [Benus et al., 1991b]. Unfortunately similar data is lacking for the other two high-aggression selection lines (TA, NC900). Nevertheless, it seems that this constant/inflexible behavior is a common feature in the SAL mice.

The overall invariance seen in the SAL mice as discussed above is likely to be reflective of a highly intrinsically organized behavior. It is possible that the SAL mice may not adequately process detailed sensory information but might revert to a previously established central motor program [Fentress, 1976]. Sensory input is essential for normal social communication; it helps in context assessment as well as agonistic behavioral modulation. In the SAL mice, a lack of adherence to ritualistic introductory behavior preceding offense, especially attack is evident right from day 1 (*social exploration* is not predictive of *threat*; *threat* is not predictive of *attack* for example). Once triggered, SAL males show many uninterrupted *attack* ↔ *chase* transitions. The further lack of release from offense was witnessed in the SAL mice even in the presence of various other likely interfering external stimuli like increasing light intensity or noise from the experimenters. Further studies are required to assess the possible sensory impairments in the SAL mice.

Thus, the initial findings of Benus et al. [1990] associating high aggression in general with inflexibility is further iterated to invariant and exclusive offense-oriented violent behavior in the SAL mice. At this point of time, it is tempting to consider the possibility that this inflexibility and a lack of social adaptive capacity might be jointly associated in the development of violence. Further behavioral and neurobiological validations will be required to assess the nature of adulthood violence and its likely convergence with inflexibility. Additionally, future studies will also focus on the temporal development of violence during adolescent or pre-adolescent periods in the SAL mice.

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REFERENCES

- Benus RF, Den Daas S, Koolhaas JM, van Oortmerssen GA. 1990. Routine formation and flexibility in social and non-social behavior of aggressive and non-aggressive male mice. *Behaviour* 112:176–193.
- Benus RF, Bohus B, Koolhaas JM, van Oortmerssen GA. 1991a. Heritable variation for aggression as a reflection of individual coping strategies. *Behaviour* 47:1008–1019.
- Benus RF, Bohus B, Koolhaas JM, van Oortmerssen GA. 1991b. Behavioral differences between artificially selected aggressive and non-aggressive mice: Response to apomorphine. *Behav Brain Res* 43:203–208.
- Caramaschi D, de Boer SF, de Vries H, Koolhaas JM. 2008. Development of violence in mice through repeated victory along with changes in prefrontal cortex neurochemistry. *Behav Brain Res* 189:263–272.
- de Boer SF, Koolhaas JM. 2005. 5-HT1A and 5-HT1B receptor agonists and aggression: Pharmacological challenge of the serotonin deficiency hypothesis. *Eur J Pharmacol* 526:125–139.
- de Vries H, Netto WJ, Hanegraaf PLH. 1993a. MatMan: A program for the analysis of sociometric matrices and behavioural transition matrices. *Behaviour* 125:157–175.
- de Vries H. 1993b. The row-wise correlation between two proximity matrices and the partial row-wise correlation. *Psychometrika* 58:53–69.
- Fentress JC. 1976. Dynamic boundaries of patterned behavior: Interaction and self-organization. In: Bateson PPG, Hinde RA (eds.). *Growing Points in Ethology*. London, UK: Cambridge University Press, pp 35–69.
- Koolhaas JM, Schuurman T, Wiepkema PR. 1980. The organization of intraspecific agonistic behaviour in the rat. *Prog Neurobiol* 15:247–268.
- Krug EG, Dahlberg LL, Mercy JA, Zwi AB, Lozane R. 2002. *World Report on Violence and Health*. Geneva, Switzerland: World Health Organization.
- Kudryavtseva NN. 2000. Experimental approach to the study of learned aggression. *Aggr Behav* 26:241–256.
- Kudryavtseva NN, Bondar NP, Alekseyenko OV. 2000. Behavioral correlates of learned aggression. *Aggr Behav* 26:386–400.
- McSweeney FK, Swindell S. 2002. Common processes may contribute to extinction and habituation. *J Gen Psychol* 129:364–400.
- Natarajan D, de Vries H, Saaltink D, de Boer SF, Koolhaas JM. 2009. Delineation of violence from functional aggression in mice: An ethological approach. *Behav Genet* 39:73–90.
- Van Hooff JARAM. 1982. Categories and sequences of behaviour: Methods of description and analysis. In: Scherer KR, Ekman P (eds.). *Handbook of Methods in Nonverbal Behaviour Research*. Cambridge, UK: University Press, pp 362–439.
- Winslow JT, Camacho F. 1995. Cholinergic modulation of a decrement in social investigation following repeated contacts in mice. *Psychopharmacology (Berl)* 121:164–172.
- Winslow JT, Miczek KA. 1983. Habituation of aggression in mice: Pharmacological evidence of catecholaminergic and serotonergic mediation. *Psychopharmacology (Berl)* 81:286–291.